Clopyralid Sampling & Testing Report

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King County
Department of
Natural Resources and Parks

Seattle Public Utilities

Report prepared by: **King County Solid Waste Division**
King County Local Hazardous Waste Management Program
Seattle Public Utility Resource Management Planning Section

Sample Collection: **Clayton Group Services**
Clopyralid Analysis: **Morse Labs, Anatek, STL**
Bioassay Analysis: **WSU Pullman, WSU Puyallup, UW Botany**

Disclaimer

The samples tested were collected in February and March of 2002 and do not necessarily reflect the current status of the presence of clopyralid in feedstocks and products available in King County. The purpose of this study was to determine if clopyralid was present in tested materials at levels sufficient to cause observable toxicity in sensitive plants and to assess the usability and comparability of clopyralid data. This report does not necessarily represent current testing performance for clopyralid and phytotoxicity by participating laboratories. This report is not intended to serve as an exhaustive study of the presence of clopyralid in the King County region.

Executive Summary

King County and the City of Seattle are concerned that clopyralid, the active ingredient in many lawn, turf and agricultural herbicides, may be present in locally produced compost products and organic feedstocks. The presence of clopyralid in compost has implications to the strength of the compost markets. Strong markets are essential to the success of recycling programs. Many King County and City of Seattle programs and initiatives are at risk with clopyralid tainted compost.

A sampling and testing study was initiated in February and March of 2002. The intent of the testing was to augment testing done by others, such as the Washington State Department of Agriculture, Washington State Department Ecology and compost producers. Additionally testing was done to provide information for the current statewide regulatory process to protect organic waste streams from contamination.

The following conclusions and recommendations are drawn from this study:

- Clopyralid was detected in laboratory analysis, and clopyralid damage was observed in bioassay testing;
- Wide variation in detection was reported among and between analytical and bioassay findings;
- A single threshold for plant damage in all materials remains unclear since thresholds appear to vary by matrix and even within a given matrix;
- Clear objectives for testing need to be established. Without clear objectives is it difficult to decide what the best testing approach should be.

There are no Standard Reference Materials (SRM) available at this time for clopyralid. This diminishes the ability of all involved in this work to truly discern data quality. A key objective of this testing is to provide information regarding the usability and comparability of clopyralid data generated from different participating laboratories. This information will be used to improve data comparability within the region.

The evidence of this study suggests that a clear, standardized analytical and bioassay methodology and testing protocol should be developed to improve confidence and comparability when discussing clopyralid presence or lack thereof in compost and other organic products and feedstocks.

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Technical Appendix II

The following materials are not included as part of this report but available on request.*

E. A 4/10/02 letter from Clayton Group Services summarizing deviations from the sampling plan
F. Data Packages from all of the laboratories involved: Morse Analytical, Anatek, STL Seattle
G. Data Packages from WSU Puyallup and WSU Pullman
H. E-mail summarizing plant toxicity results from Doug Ewing of the University of Washington Department of Botany and Clopyralid Bioassay Draft Procedure
I. *Testing results for clopyralid and picloram in compost products and related materials from King County*, prepared by King County Solid Waste Division
J. Organic data review: Clopyralid Data Review Completions Sheet and associated data review narrative, for Morse, Anatek and STL Laboratories. Prepared by Dana Walker, Organic Chemist, King County Environmental Laboratory.
K. Bioassay data review: prepared by Fran Sweeney, Toxicologist, King County Environmental Laboratory

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I. Background

King County and the City of Seattle are concerned that clopyralid, the active ingredient in many lawn, turf and agricultural herbicides, may be present in locally produced compost products and organic feedstocks (such as lawn trimmings, manure's, straw, hay and Christmas trees). Clopyralid does not readily break down during the composting process and is damaging to sensitive plant species such as peas, beans and tomatoes at trace levels. Two composting facilities in Eastern Washington (City of Spokane's and WSU's in Pullman) have not been able to sell compost products for more then two years due to complaints from commercial and residential customers of vegetable crop failures attributable to clopyralid levels (and picloram in the case of WSU).

The Washington State Department of Agriculture (WSDA) sampled compost products and feedstocks around the State in October 2001 and found clopyralid present in almost all samples. WSDA has adopted a Permanent Rule effective July 2002 which prohibits the use of clopyralid-containing herbicides for lawn and turf use with an exception provided for golf courses which do not send grass clippings, leaves or pruning material to a composting facility. WSDA will determine the effectiveness of this rule through further sampling and testing of compost and feedstocks.

Many compost facility operators are currently conducting bioassays on batches of compost to ensure that clopyralid-tainted product is not sold to the general public for gardening purposes this year. However, a range of bioassay techniques is available which could yield differing results. Likewise, a number of laboratories now offer analytical services for clopyralid. The detection limits and methodologies employed by these labs vary. In order to provide assurance to the gardening public that compost products are being tested accurately, standardization of bioassay techniques and lab analytical methods would be beneficial.

The presence of clopyralid in compost has implications to the strength of the compost markets. Strong markets are essential to the success of recycling programs. The following King County and City of Seattle programs and initiatives are at risk with clopyralid tainted compost:

- Disposal ban on yard trimmings in curbside collected garbage and at the landfill;
- Centralized yard debris collection and processing;
- Backyard Composting programs;
- Soils for Salmon and other compost marketing initiatives linking improved soils with improvements in water quality;
- Pesticide reduction strategies;
- Best management practices for manure in order to improve water quality.

II. Goals of Clopyralid Sampling/Testing Study

Data was collected to address the following questions:

1. Is clopyralid present in the tested materials at sufficient levels to cause observable toxicity in sensitive plants?

2. Is there a correlation between clopyralid concentrations and the results of the bioassay for the tested materials?

3. How comparable are the data being generated from different participating laboratories?

These purposes are initially stated in the project sampling plan and data collection was designed to inform these questions.

The intent of the testing is to augment testing done by others such as the Washington State Department of Agriculture (WSDA), Washington State Department of Ecology and compost producers and to provide information into the regulatory process underway to protect organic waste streams from contamination. The approaches employed in much of this prior testing have not been coordinated among testing entities. Results from this study may aid future coordination of testing.

III. Methodology

King County and the City of Seattle selected materials to be sampled. These are summarized in the table below:

Table 1: Sampled Materials

Sample ID*	Composition
1	Compost - yard waste, vegetable trimmings and spent coffee grounds
2	Composted steer and chicken manure (2.5:1) and aged bark
3	100% recycled yardwaste and sand.
4	Compost - 100% recycled yardwaste
5	Compost - yard waste, vegetable trimmings and spent coffee grounds
6	Compost - yard waste, vegetable trimmings and spent coffee grounds
7	Composted dairy manure and sawdust (1:2)
8	Sandy loam soil, compost (100% recycled yardwaste) and peat moss
9	Wood chips from Christmas trees
10	Straw (from Quincy area)

These ten samples of both bulk and bagged compost feed stocks and products were collected randomly by consultants over a two-week period in February and March of 2001 for clopyralid and picloram analysis.

Samples, including six blind replicates, were then split among participating laboratories, listed below. A summary of sampling shipments, in table form, appears in the appendices. Clopyralid laboratories were selected to achieve low detection limits for clopyralid (1-5 ppb). Bioassay laboratories were selected largely due to prior participation in this testing, under the hire of others. The three-bioassay laboratories are all academic institutions offering differing bioassay analysis.

The participating analytical laboratories were:

- Anatek (Moscow, Idaho) which has analyzed clopyralid in compost and other matrices extensively over the past two years;
- Morse Lab (Sacramento, California) which has recently developed the ability to detect clopyralid in grass clippings and compost; and,
- STL (Fife, Washington) which offers clopyralid analyses for all matrices.

The primary participating academic institutions for the bioassay part of the study were:

- Washington State University in Pullman (which has done extensive bioassay work for WSU's composting facility), and
- Washington State University in Puyallup (which has conducted clopyralid dissipation studies in turf).

Additional testing for bioassays was conducted by the Botany Greenhouse at the University of Washington and, at a later date, by WSU Pullman using a different protocol.

Caution must be used when using data from these additional tests. Neither the UW Botany Lab nor the June Pullman work was included in the original Sampling Plan or subsequent Quality assurance review.

Quality Assurance review was conducted by King County staff as an initial step in preparing this report. This work is summarized in the reports listed in the appendices. Notable observations made in this Quality Assurance review are summarized in this report.

IV. Findings

Clopyralid Results:

Clopyralid data are presented in the table below. Variation is notable among reported results, including detection limits. Clopyralid was found in all six yard debris compost and mulch products sampled when the analysis was performed by Anatek and Morse. Likewise there was low (3 ppm) to no clopyralid detected in the composted manure products according to the two labs (Anatek and STL) which received those samples.

Clopyralid was not detected in the two feedstock samples collected of Christmas tree chips or straw. Similarly, picloram was not reported in any sample. Anatek and STL tested for picloram. It was important to screen for picloram, a related chemical, because of its ability to influence bioassay results.

Table 2: Clopyralid results

Sample ID	Composition	Anatek	Morse	STL
1	Compost - yard waste, vegetable trimmings and spent coffee grounds	9	16.3, 18.8	
6	As above	17	43.1	
5	As above	15	25.4	
4	Compost - 100% recycled yardwaste	18	28.7	
3	Compost - 100% recycled yardwaste and sand.	12		ND <1.88
8	Sandy loam soil, compost (100% recycled yardwaste) and peat moss	12		ND <1.78
2	Composted steer and chicken manure (2.5:1) and aged bark	3		ND <3.08, <2.39
7	Composted dairy manure and sawdust (1:2)	ND <1		ND <4.22
9	Wood chips	ND <1		ND <4.34
10	Straw (from Quincy area)	ND <1		ND <2.1

- All results in ppb, on a dry weight basis
- All samples except 9 and 10 contain compost
- No sample was tested by all three laboratories
- Blank spaces indicate a sample was not tested by this laboratory
- ND Reported figure is the PQL provided by the laboratory
- The STL PQL varies for each sample

Clopyralid Data Quality and Comparability

The data review conducted for clopyralid was limited by the types of information available. The following important elements of data review were not available:

- a copy of the method,
- the result of an SRM analysis, and
- complete access to all data records.

Additionally, a laboratory site inspection was not included in this study. A site inspection can yield additional information. However, the laboratories provided full data packages. These packages were requested to provide sufficient information to "validate" the results, or reproduce each calculation. Also information about the methods employed was gathered during the preparation of the Sampling Plan (this information is included in the attached Sampling Plan – Appendix I-A).

There are no Standard Reference Materials (SRM) available at this time for clopyralid. This diminishes the ability of all involved in this work to truly discern data quality. A key objective of the testing is to provide information regarding the usability and comparability of clopyralid data generated from different participating laboratories. This will better allow others involved in testing to compare "apples to apples," and to develop a unified approach for clopyralid and bioassay testing.

A "first glance" indicates reasonable agreement between Anatek and Morse (see Table 2. above). However, in all cases the Morse result is higher, approximately by a factor of two. Morse appears to have used a method of quantitation similar to "isotope dilution." This method, to a large degree, "corrects" for recovery by quantitating against a *surrogate compound* added before sample extraction. Though in this case, the surrogate is referred to as an *internal standard*. This approach inherently corrects for recovery.

For testing conducted at Anatek, the internal standard is added just before instrumental analysis, not before extraction. This method of quantitation does not attempt to account for method recovery. All other things equal, this would produce higher results by Morse. And approximately a factor of two would be expected, based on Anatek's average surrogate recovery of 57%. This is the center point of their surrogate control limits.

STL did not detect clopyralid in any of six samples. This data generally agrees with Anatek for four of these six samples. (One sample has a result of 3 ppb from Anatek and < 3.08 from STL.) However, there is significant disagreement for samples 3 and 8, see Table 2. In both these samples, reported as 12 ppb by Anatek, STL did not detect clopyralid, with detection limits of approximately 2 ppb. STL data has a negative correlation with Anatek and the Puyallup pea bioassay.

This quality assurance review has not been conducted to definitively discern data quality. It has been conducted as a precursor to data use and a tool to inform method standardization efforts in the region. In general, laboratories were reasonably compliant with project instructions.

However, in all cases there were instances of deviation from instruction, or clerical errors, or a question about a particular analytical approach within a laboratory.

If the STL data are correct it would change some of the findings in this report. However, there is a large body of accumulated data collected regionally, by the manufacturer, Washington State Department of Agriculture, University extensions and municipalities that indicate a nearly ubiquitous nature to clopyralid contamination in compost. Samples reported herein were taken before the change in regulations governing clopyralid use went into effect.

Bioassay Results:

There are two groups of bioassay data in this report from a quality assurance viewpoint. The first group was planned for and reviewed under the Sampling Plan. The second group of results was generated at a later date. Discussion is separated by these two groups of data when practicable.

Data from the first group of bioassays are listed below in Table 3. These data are ranked by their response to the pea bioassay. This is because of the following reasons;

- The beans did not respond in this round of testing (failed bioassay).
- Regional testing has no "reference material" for this testing, either clopyralid or bioassay. In this void the Sampling Plan proposes to use the bioassay results as a check on the clopyralid results. It seems reasonable, based on statistical agreement between the pea bioassay and clopyralid detections, to rank results by pea bioassay response.

Table 3: Bioassay Results, Ranked by Pea Response

Sample ID	Composition	WSU Puyallup Peas (Knight)	WSU Puyallup Green Beans (Provider)	WSU Pullman Pinto Beans
6	Yard waste, vegetable trimmings and spent coffee grounds	4	0	No symptoms
1	As above	2.5	0	No symptoms
8	Sandy loam soil, compost (100% recycled yardwaste) and peat moss	2.5	0	No symptoms
3	100% recycled yardwaste and sand.	2, < 1	0,0	No symptoms
4	Compost - 100% recycled yardwaste	1	0	No symptoms, No symptoms
5	Yard waste, vegetable trimmings and spent coffee grounds	< 1, 0	0,0	No symptoms
2	Composted steer and chicken manure (2.5:1) and aged bark	0	0	No symptoms
7	Composted dairy manure and sawdust (1:2)	0	0	No symptoms
9	Wood chips	0	0	No symptoms
10	Straw (from Quincy area)	0	0	No symptoms

- WSU Puyallup: Scale 0-5 (0 no symptoms, <1 possible, 2 minor, 3-4 moderate, 5 severe symptoms.)
- WSU Pullman: Yes/No scoring system.

Among the initial group of bioassay tests, plant damage only occurred in pea bioassays.

The following table includes the second group of bioassay data. However, the ranking by pea bioassay is retained to aid interpretation.

Table 4: Bioassay results including a comparison of application rates, dilution media, and test species.

	WSU Puyallup		WSU Pullman	WSU Pullman June testing		UW - Botany Greenhouse	
Application (Ratio of test material:dilution media)	2:1 by volume (66% compost product)		1:5 by weight (20% compost product)	1:1 by volume (50% compost product)		100% test material except straw and woodchips 1:1 by volume (50% compost product)	
Dilution media	Perlite (Peat based potting mix, Sunshine #2, added if material is very porous)		Peat / perlite potting mix	Perlite		Peat based potting mix (Sunshine #4)	
Test species	Beans (Provider)	Peas (Knight)	Beans (Pinto)	Beans (Pinto)	Peas (Joel)	Peas (Green Arrow)	
SAMPLE ID	Note different scoring systems used.						
						23 days	31 days
6	0	4	No symptoms	2	0	very strong	severe
1	0	2.5	No symptoms	None	1		
8	0	2.5	No symptoms	None	0.5	strong	moderate
3	0, 0	<1, 2	No symptoms	2	2		
4	0	1	No symptoms/ No symptoms	None	0.5	no symptoms	no symptoms
5	0, 0	<1, 0	No symptoms	1	0	no symptoms	slight
2	0	0	No symptoms	None	1		
7	0	0	No symptoms				
9	0	0	No symptoms			no symptoms	no symptoms
10	0	0	No symptoms			no symptoms	no symptoms

Caution must be used when analyzing these results in relation to the other results because these tests were not included in the original Sampling Plan, did not undergo the Quality Assurance Review and underwent different storage conditions.
Blank space means sample not tested.

Explanation of Scoring Systems:

- WSU Puyallup: Scale 0-5 (0 no symptoms, <1 possible, 2 minor, 3-4 moderate, 5 severe symptoms.)
- WSU Pullman: Yes/No scoring system.
- WSU Pullman June: Scale of 0-3 (0 no symptoms, 1 slight, 2 moderate, 3 severe symptoms). None means no plants grew.
- UW Botany: Scoring system altered between the two reporting periods. Scoring system at 31 days has four levels: no symptoms, slight, moderate, and severe symptoms.

Bioassay Data Quality and Comparability

Table 5: Comparison of Bioassay procedures used in the testing

	WSU-Puyallup	WSU-Pullman	WSU Pullman June Testing	UW Botany Greenhouse	
Ratio of test material to dilution media	2:1by volume (66% compost product)	1:5 by weight (20% compost product)	1:1 by volume (50% compost product)	100% test material except straw and wood chips which were tested at 1:1 by volume (50% compost product)	
Media used	Perlite (Peat based commercial potting mix, Sunshine # 1 added if material is very porous)	Peat based commercial potting mix (Soil Conditioners)	Perlite	Peat based commercial potting mix (Sunshine #4)	
# seeds per pot	3	4		2 or 3	
# pots per species	4	3		2-3 (pots of both peas and beans - replicates were limited by the amount of test material provided)	
Pea depth & variety	1 inch / Knight	NA	Joel	1/4 inch / Green Arrow	
Bean depth & variety	1-1.5 inches / Provider	1/2 inch / Pinto	1/2 inch / Pinto	1/4 inch / Contender	
Controls	Negative - Sunshine # 1 only. Positive - Known contaminated compost	Negative - Potting soil only. Positive - 2 herbicide contaminated composts (4 & 51 ppb)		Negative - Sunshine # 4	
Light regime	Artificial light 12hrs/day	Artificial light 12hrs/day	Artificial light 12hrs/day	Artificial and natural 16hrs/day	
Irrigation regime	Automatic watering twice/day. Attempted to limit flushing to prevent loss of clopyralid via leaching.	Watered by hand, 3 times/wk	Watered by hand, 3 times/wk	Watered carefully & saucered to minimize leaching. Watered with clear water until germination, with a complete fertilizer after germination.	
Pot spacing	Spaced far enough apart to prevent cross contamination from watering.			Saucered so cross contamination from watering not an issue.	
Fertilizer regime	None applied	8 oz. Osmocote mini 18-5-9/cubic foot	None applied	Watered with a complete fertilizer after germination.	
Temperature regime (F)	65 - 75			55 - 75	
Test duration	21 days	21 days	21 days	23 days	31 days
Scoring system	Scaled 0-5: Zero=no symptoms and 5=severe symptoms.	Yes/No	Scaled 0-3: Zero=no symptoms and 3=severe symptoms.		Four levels: No symptoms, slight, moderate, severe

- Shaded area indicates bioassays not included in the Sampling Plan.
- Blank space means information not reported.

Procedures used during all four bioassays are compared above in Table 5. Variations between procedures were numerous, though some have greater significance than others. The variations of most concern are ratio of test material to media, test species used, scoring system and irrigation regime.

It should also be pointed out the second round of bioassay testing was conducted under different sample storage conditions, and this may have an effect on data. This includes different lengths of storage time, different temperatures and freezing and thawing.

Ratios of test material to media varied greatly, as detailed in Table 5 and below. No procedures used a range of ratios, an approach that is commonly recognized, to quantify upper and lower limits of phytotoxicity. Additionally, ratio of test material to media was proportioned either by volume or by weight. While media remained a constant density, the test material included compost, manure-based compost, wood chips and straw. This variety in test material density confounds interpretation of test material dilution. None of the dilution techniques, either by volume or be weight attempt to analyze test material at a standard percent, by dry weight. For example, the first round of Pullman bean bioassays report that samples are diluted by mass, 1:5, to result in a nominal test concentration of 20% by weight. However, percent moisture correction indicates that the test "pots" contain from 11.4 to 24.4 percent test material by weight.

Applications levels for compost in bioassay trails:

WSU Puyallup: 67% compost with 33% perlite on a volume basis with no fertilizer added.

WSU Pullman: 20% compost to 80% potting mix on a weight basis with fertilizer added to rule out any confounding nutrient imbalance. The 25% compost is generally the recommended application rate for compost use in order to avoid any other complicating phytotoxic effects if the compost is not stable.

WSU Pullman in June: 50% compost to 50% perlite on a volume basis with no fertilizer added.

UW Botany Lab: 100% compost (except for wood chips and straw where 50% compost was used because no germination occurred in 100% compost) with fertilizer added after germination.

Test species used were inconsistent between institutions. While bioassays from the first round indicate that peas may be more sensitive than beans, other variables confound interpretation.

Each institution used a different Visual Assessment scoring system and WSU Pullman used a different scoring system for round one and round two of bioassay testing. The ranked scoring systems appear to be more appropriate as they provide an indication of the severity of effects and allow for ranking of samples within a study. ASTM Standards for conducting terrestrial plant toxicity tests also use a numerical scale (0 to 4) for scoring visual effects.

Variations in irrigation regimes are worth noting. UW Botany expressed concerns about clopyralid's ability to leach from the compost/media matrix and their solution was to saucer all pots. Although WSU Puyallup did not expressly state a concern with the potential of clopyralid to leach, they did state that they attempted to balance irrigation inputs with transpiration.

Correlation of Bioassay and Clopyralid Results:

Again, the two groups of bioassay data are separated in discussion. The first round of bioassays is discussed immediately below. Again, should the STL clopyralid non-detects in samples 3 and 8 be correct, these findings would change.

Six of the ten samples damaged pea plants. These samples contained the six highest detections of clopyralid, as shown in Table 6 below. However, the plant damage scores do not correlate directly with the detected clopyralid. The fifth and sixth plant damage scores correspond to the second and third clopyralid rankings. This is reflected in statistical findings.

A statistically significant correlation could be found between the Puyallup pea bioassay and the Anatek laboratory findings. There was less of a statistical correlation between the Morse data and the Puyallup pea bioassay. The STL data is negatively correlated with Anatek and the Puyallup pea bioassay. In summary, when clopyralid increases toxicity increases, though a clear 1:1 relationship is not present. It is notable that a clopyralid result as high as 25 ppb has a "0" effects reported in the pea bioassay.

The second group of bioassays has not been analyzed statistically. Visually, these samples show an uneven correlation with clopyralid results, perhaps more so than the first group of data.

Data in the table below are ranked by WSU Puyallup pea bioassay.

Table 6: Comparison of pea bioassay data with clopyralid detection's

SAMPLE ID	WSU Puyallup	WSU Pullman June testing	UW Botany Greenhouse		Anatek ppb (PQL <1)	Morse ppb
			23 days	31 days		
6	4	0	Very strong	Severe	17	43.1
1	2.5	1			9	16.3, 18.8
8	2.5	0.5	Strong	Moderate	12	
3	<1, 2	2			12	
4	1	0.5	No symptoms	No symptoms	18	28.7
5	<1, 0	0	No symptoms	Slight	15	25.4
2	0	1			3	
7	0				ND <1	
8	0		No symptoms	No symptoms	ND <1	
9	0		No symptoms	No symptoms	ND <1	

Caution must be used when analyzing these results in relation to the other results because these tests were not included in the original Sampling Plan, did not undergo the Quality Assurance Review and underwent different storage conditions.

- Blank space means sample not tested.

- All clopyralid results are ppb dry weight
- WSU Puyallup: Scale 0-5 (0 no symptoms, <1 possible, 2 minor, 3-4 moderate, 5 severe symptoms.)
- WSU Pullman June: Scale of 0-3 (0 no symptoms, 1 slight, 2 moderate, 3 severe symptoms).
- UW Botany: Scoring system altered between the two test duration's. Scoring system at 31 days has four levels: no symptoms, slight, moderate, and severe symptoms

V. CONCLUSIONS and RECOMMENDATIONS

Conclusions:

This King County and Seattle Clopyralid Testing Study addressed three questions. Based on information presented herein, the best answers at this time are as follows:

1. Is clopyralid present in the tested materials at sufficient levels to cause observable toxicity in plants?

A statistical correlation was established between clopyralid and toxicity detections in this study. Establishing the "level" at which that occurs is more involved. A previous series of tests conducted at Pullman (Appendix I-D) and regional discussion of those tests indicate that bioassay response occurs at the low ppb level. For this study, bioassay thresholds seemed to occur at higher levels. This includes a clopyralid result of 25.4 ppb associated with a negative pea bioassay.

Close examination of the Pullman spreadsheet indicates that there is not a single compost sample in the Pullman spreadsheet that meets the following conditions:

- a failed bioassay (no symptoms observed)
- a clopyralid concentration of < 40 ppb
- a non detect for picloram (picloram is even more toxic to plants than clopyralid)

A sample that meets these three conditions would establish the possibility that clopyralid in compost at less than 40 ppb causes plant bioassay failure. It would also be beneficial to view bioassay data that passes, to provide insight into the bioassay threshold. There are no compost samples on the Pullman spreadsheet that pass. The bioassay failures below 10 ppb on the Pullman spreadsheet are all in non-compost matrices.

Close examination of the King County/Seattle data raises the possibility that damage thresholds may differ for different matrices. *Indeed, each material may have it's own damage threshold.*

Four of the ten samples tested in this study passed the initial pea bioassay, perhaps the most sensitive bioassay, so in these instances clopyralid was not present at harmful levels. Where initial bioassay tests failed the clopyralid detections ranged from 9-18 ppb (Anatek) and 16-43 ppb (Morse). Bioassay testing at a later date at Pullman shows effects in a 3 ppb sample (Anatek).

2. Is there a correlation between clopyralid concentrations and the bioassay results for the tested materials?

This has, to a large extent, been discussed above. Apparent differences in bioassay thresholds may be due to the ability of the chemical and biological methods to "access" the clopyralid present in the samples. Even when accounting for differences in the laboratory results, some matrices, such as manure, appear to have lower thresholds for bioassay failure than compost. This could be due to clopyralid "hiding" in wood particles in some matrices during bioassay testing.

3A. How comparable are the data being generated from different participating laboratories which are using different analytical methods for clopyralid analysis? (For ease of discussion, reader note that question 3A and 3B derive from a single data objective)

Within this study, data comparability is discussed above. There are notable differences in results between laboratories.

Data from this study appears to show a higher threshold for plant toxicity than implied on the Pullman spreadsheet. This may be due to matrix effects or the use of differing methods and laboratories.

3B. How comparable are the bioassay results being from different participating academic institutions that are using different protocols?

During the initial bioassay testing, notable variability was observed in results. This continued in the second round of testing.

Ratios of test material to media may explain some of the differences in results but because the lowest ratio (20%) was only tested on beans this is impossible to verify. Despite differences in results, these appear to be due to method differences and not data quality issues.

A regional approach to bioassay testing should address the following elements of bioassay testing:

- test species: which species and number of species
- sample preparation, i.e. sieving
- dilution of test sample
- accounting for wet weight
- dilution material
- test duration
- scoring system
- watering regime

Recommendations:

Given wide variation in clopyralid findings in this study and conflicting reports from other data, it is apparent that a standardized analytical lab and bioassay methodology would be beneficial to the composting industry, growers, regulators and compost consumers. There is no Standard Reference Materials (SRM) available to evaluate the ability of analytical labs to determine the true value of clopyralid in a given sample matrix at this time. The analytical labs involved in this study were also operating under different Quality Assurance systems.

The findings in this report have produced the following recommendations:

- WSDA and Ecology convene a testing protocol forum for both analytical and bioassay methods to address variation in methodology and develop a regional standard reference material for clopyralid analytical and bioassay testing.

- A system for data sharing should be established with more sharing of data.

- Access to methodologies, both clopyralid and bioassay, would enhance efforts to standardize testing.

- Lab certification for all testing, clopyralid and bioassays, may be appropriate. This may be a formal certification by an agency certifying body or an informal "round robin" study where labs demonstrate competency.

- Development of Instructions for testing laboratories.

- Efforts at method standardization should consider the following findings from this report.

    - Peas appear to be more sensitive to clopyralid than beans.

    - The bioavailability of clopyralid may be the cause of different bioassay results. Specifically, clopyralid may "hide" in wood particles in some matrices. Both further testing of the "woody fraction" and data sharing among all parties seems appropriate.

- An agreement among testing entities (for example, producers, users, regulatory agencies) be developed so that decisions made to standardize testing approach are followed regionally.

Technical Appendix I

Appendix I-A

Quality Assurance Review
George Perry
King County Local Hazardous Waste Management Program

The term *quality assurance* refers to the sum total of systems that guide or affect an activity. The objective of quality assurance is to ensure that a product or service satisfies the demands of it's use, either stated or implied. (E-4, 1994)

Therefore, this review focuses on the ability of this data to meet intended uses. These intended uses are summarized in the Sampling Plan. Some important quality assurance concepts are summarized below, to provide a foundation for following discussion.

Method defined parameter
Method defined parameter is a commonly recognized quality assurance phrase used by the US EPA. It refers to a parameter which is defined by the analytical method and not by the laws of science.

An example of a method-defined parameter is *dissolved lead*. A statement in the Federal Register defines dissolved lead as passing through a 0.45 micron filter.

Conversely, *total lead* is not method defined. Lead is defined in the periodic table and a *single numerical value* represents it's concentration in a given sample.

Similarly, plant toxicity for this study is method defined. Toxicity was determined by planting legumes and determining if growth was *normal*. The dosage, the test species, sample handling and even normal growth are defined by the method.

Conversely, the concentration of Clopyralid in compost is not method defined. Clopyralid is a molecule with a specific structure and a registered CAS number, CAS # 1702-17-6. A given sample has a *single numerical value* of clopyralid. The ideal objective of testing is to determine that single numerical value.

SRM
Standard reference materials (SRM) establish the ability of a laboratory to determine the *true value* of a parameter in a given sample matrix. For our purposes, the true value is synonymous with the *single numerical value* discussed above.

SRM true values are not method defined. To the best ability of science, using at least two *entirely different methods*, the true value is determined. NIST uses an extremely rigorous working definition of "two different methods". For example, lead would not be determined by

two different EPA atomic absorption methods for lead. Instead, a spectrophotometer and x-ray diffraction might be used. There is no Code in the Federal Register, or existing method that produces a recognized result. The reported *true value* represents the best attempts of science to obtain the scientifically based *single numerical value* noted above.

As an example, a marine sediment which contains lead is sold by NIST (National Institute of Standards and Technology) with a certificate of analysis, which includes the lead concentration (true value). This marine sediment is taken from the marine environment, homogenized, tested for homogeneity, analyzed by at least two methods, tested for stability and released for sale. It is used by laboratories testing marine sediments for lead to demonstrate their ability to obtain accurate results when faced with the unique challenges posed by testing marine sediments.

There are no SRM currently available for clopyralid.

There would not be any potential for a plant toxicity SRM to be available, because toxicity is method defined.

QA systems

Published methods are commonly created under the umbrella of a QA system. US EPA 8151A (clopyralid) is contained within the RCRA SW 846 compendium of methods. The DOW clopyralid method has been created under Good Laboratory Practice (GLP). This QA system is commonly applied to TSCA (Toxic Substances Control Act) and FIFRA (Federal Insecticide, Fungicide and Redenticide Act) studies. These QA systems could be construed as having different objectives.

GLP methods are tested under the conditions of use. Before reporting a single result the laboratory must go through a matrix specific "certification". Method performance is demonstrated over the expected concentration range. Rigor is maintained throughout data collection, for example a sample result above the certification range initiates a supplementary certification demonstrating method performance at the higher level. The objective, stated or implied, when testing under GLP is to approach the *true value*.

Conversely, RCRA SW 846 is developed for multiple analytes in all matrices. The method is not rigorously tested under each condition of use. Instead, the objective is to report results which meet QC criteria specified in the method. For example, surrogate recovery limits are established statistically over time using data from a wide variety of sample matrices. These control limits can appear lenient, depending on the nature of the study. A recovery of 100%, seemingly perfect, can be out of control and reject the data.

Neither of these systems is "better" than the other. They each serve their intended use. However, as we compare data from multiple QA systems, these distinctions will become important.

Real recovery and sample processing

The term *sample matrix* has been used throughout this report. At a gross level, samples are commonly categorized as liquid, solid, oil, or tissue. More detailed categorization might include the terms soil, compost, grass clippings.

Samples may be taken out of the jar and tested without alteration. However, some labs may have stricter criteria, which result in grinding to reduce particle size.

Many of the samples tested in this study are compost. Compost contains a variety of materials and particle sizes. These properties, along with percent moisture, vary from product to product. Contaminants such as clopyralid may/or may not be distributed throughout a sample matrix homogeneously

Within soil, clopyralid has been determined to not readily adsorb to soil particles (US EPA 1986). However, in the event of clopyralid use on landscape plants, it is likely that the clopyralid is incorporated within the woody tissue.

Keeping these principles in mind, samples that are a mixture of soil and composted woody material are then tested for clopyralid or plant toxicity. Depending on the distribution of clopyralid within the sample matrix, one or both of these methods could experience difficulty "accessing" some or all of clopyralid in the sample.

QC Samples

In general, all QC samples fall into one of two categories, positive or negative control. These samples demonstrate that a parameter will be detected if present, and not detected if not present. Quality control samples also provide important information about the accuracy and precision of analytical data. A summary of QC sample types is listed in the table below. Sample replicates do not fit neatly in either category are listed in both.

positive control	**negative control**
the following applies to bioassay: positive control sample replicate	the following applies to bioassay: negative control sample replicate
the following applies to clopyralid: matrix spike blank spike surrogate compound blank spike SRM (if available) sample replicate	the following applies to clopyralid: method blank sample replicate

Combined with a review of sample analysis documentation, and procedures, these QC samples give an even greater understanding of method performance. It is important to fully understand just what these samples do and do not provide.

For example, matrix spikes and surrogates are added to the sample early in the analysis. They are not homogeneously distributed throughout the sample matrix. So a good clopyralid matrix spike recovery, say 75%, implies that the method operated at 75% recovery from the point the spike was added. It says nothing about the ability of the sample extraction to access all of the clopyralid present. Indeed, method 8151A specifies that the surrogates and matrix spike compounds be added to the initial extraction solution, they never come into direct contact with the sample itself.

Similarly, a "non-detect" in a method blank does not mean all the samples were not contaminated. It means the blank was not contaminated. It is a good start in understanding whether positive bias has occurred.

Ideally, for example, to eliminate positive bias as a concern when testing for clopyralid:

- the blank would be clean,
- matrix spikes, blank spikes, surrogates and SRM are within control limits,
- the laboratory would employ a rigorous cleaning procedure,
- all lots of reagents are checked for contamination prior to use,
- an additional method blank is performed if a new reagent lot # is employed,
- sample chromatograms indicate a lack of positive interference, or the instrumental ability to accurately exclude this interference from the result,
- the analytical standard is accurate and the instrument maintains calibration specifications.

An SRM measures method performance over the entire spectrum of sample analysis. Assuming the matrix and concentration are relevant, no other positive control QC sample inspires the confidence in data that an SRM provides.

Sample Management

Sample management refers to the systems used to identify, ship and store samples and document these steps. Sample collection was documented using the *Sampling Documentation* form contained in the Sampling Plan. COC documentation was also initiated by the consultant at the time of sampling.

Diversions from the sampling plan were implemented and have been documented below:

COC's

Transfer of custody was documented among the consultant employees. However, COC were not filled out correctly, as the transfer of samples to the laboratories from the consultant is not "relinquished" on the COC. Completed COC were only received from Anatek and STL initially. Morse COC were obtained by visiting the consultant on 5/2/02. WSU Pullman COC were obtained after a phone request. WSU Puyallup original COC have been requested.

Shipping

Samples were shipped using FedEx *Standard Overnight*. However, the *Standard Overnight* box is not checked on the 3/7/02 shipment to WSU Puyallup.

Samples were shipped in batches to contract laboratories along with COC. Agreement among shipping papers and sample documentation has been checked for the following:

- does the date of receipt at the laboratory agree with the shipping papers
- are the samples reported by the laboratory accurately listed on the COC
- are the sample numbers in agreement with sample numbers assigned at the time of sampling

Clerical Errors

Clerical errors have occurred with sample ID. However, the unique nature of the sample ID, with seemingly random assortments of letters and numbers, facilitates clarification of these issues. For any given sample reported by the laboratory, the possibility of a clerical error (misidentification) seems remote, due to the uniqueness of each sample ID.

Also, a sequential number from 1 through 10, exists at the end of each sample ID#, and there has been no question regarding this portion of the sample ID#. Additionally, date of sampling corroborates that sample identification is correct.

The consultant used apostrophes in some sample ID's, we have elected to drop these from our data handling efforts. The consultant has complied with our request to no longer use apostrophes in sample identification.

Errors and inconsistencies were present in the body of sample documentation. However, the body of sample documentation contains sufficient information to document sample custody.

In summary, clerical errors were encountered in the sample identification system. However, no instances were encountered that called into question the identity of a sample.

Sample Retention

Laboratories were requested to retain samples until permission to discard was provided.

Data Handling

Date have been used without alteration, except for the following instances:

- Morse reported wet weight data in as many as eight significant digits. These data were rounded to four digits, calculated to a dry weight basis using percent moisture data provided by Morse and reported in three digits.
- *In the following instance, data were evaluated and a decision made to not alter the data:* Sample CSTR10-C was reported as ND for both clopyralid and picloram by Anatek, with a PQL of 1 ppb. The PQL is not corrected for percent moisture. Available percent moisture data from WSU -Pullman reports this sample as 5% moisture. Or 95% solids. This is a high percent solids number and therefore the Anatek data was used without alteration.

References

ANSI/ASQC E4-1994 Specifications and Guidelines for Quality Systems for Environmental Data Collection and Environmental Technology Programs. 1994 ASQC, Milwaukee, Wisconsin

1986 Pesticide Fact Sheet, Clopyralid. US EPA, Washington DC

Summary of Sample Shipments

Destination	Date of Sample Shipment	Date Received and Samples	Sampling Date	Comments
Anatek	2/28/02	3/01/02 receipt		all Anatek reported in one data package.
		MSCG01-C	2/25/02	COC in package agree with reported samples.
		MSWF02-C	2/25/02	
		PTEM03-C	2/25/02	
		SCC04-C	2/26/02	
		CGBC05-C	2/27/02	
Anatek	3/07/02	3/11/02 receipt		all Anatek reported in one data package. including shipment above.
		LSCGC06-C	2/27/02	
		SM07-C	3/04/02	PT3W08 reported as PT0308. Also identified as PT0308 on COC.
		PT3W08-C	3/04/02	
		CFCTC09-C	3/05/02	
		CSTR10-C	3/05/02	COC in package agree with reported samples.
Morse	3/11/02	3/12/02 receipt		one data package. no COC contained. signed copies obtained from Clayton.
		MSCG01-D	2/25/02	
		MSCG01-F	2/25/02	reported samples do not agree with Clayton COC.
		SCC04-D	2/26/02	Morse COC documents sample custody within lab.
		CGBC05-D	2/27/02	
		LSCGC06-E	2/27/02	
STL	3/06/02	3/07/02 receipt		four data packages. contains returned COC.
		MSWF02-D	2/25/02	COC in package agree with reported samples.
		PTEM03-D	2/25/02	
		MSWF02-E	2/25/02	
		SM07-G	3/04/02	
		PT3W08-G	3/04/02	
		CFCTC-09-G	3/05/02	
		CSTR-10-G	3/05/02	
WSU-Pullman	2/28/02 & 3/07/02	unknown receipt at this time		
			2/25/02	sample PT3W08-A was reported as PTEW08-A.
		MSCG01-A	2/25/02	Sampling date is correct.
		MSWF02-A	2/25/02	
		PTEM03-A	2/26/02	
		SCC04-E	2/27/02	
		CGBC05-A	2/26/02	
		SCC04-A	2/27/02	
		LSCGC06-A	3/04/02	
		SM07-A	3/04/02	

		PTEW08-A CFCTC09-A CSTR10-A	3/05/02 3/05/02	
WSU-Puyallup	2/28/02 & 3/07/02 & 3/11/02	unknown receipt at this time MSCG01-B MSWF02-B PTEM03-B PTEM03-E SCC04-B LSCGC06-B CGBC05-B CGBC05-E SM07-B PT3W08-B CFCTC09-B CSTR10-B	 2/25/02 2/25/05 2/25/02 2/25/02 2/26/02 2/27/02 2/27/02 2/27/02 3/04/02 3/04/02 3/05/02 3/05/02	COC have been requested.

Appendix 1-B

Summary of The Statistical Analysis of The Data Matrix
Prepared by King County

Spearman correlations have been used to compare data since some of the data, the UW Botany definitely, and the Pea data, most probably, are categorical in nature rather than continuous.

For each comparison, the correlation is followed by the confidence level. All clopyralid data is included in the comparisons. Detection limits have been divided by 2. All bioassay data from the first round of testing are included.

For all tests the average of replicates has been used.

Statistics were analyzed for the short duration bioassay at UW school of botany. They are not reported here as these samples were stored under differing conditions from the first round of bioassay testing.

Sprearman Correlation Coefficients

	Anatek	Morse	STL	Puyallup Pea
Anatek	NA	0.77 not significant at the 90% confidence level	negative correlation	0.755 significant at the 95 % confidence level
Morse	0.77 not significant at the 90% confidence level	NA	no common data, not compared	0.6 not significant at the 90 % confidence level
STL	negative correlation	no common data, not compared	NA	negative correlation
Puyallup Pea	0.755 significant at the 95 % confidence level	0.6 not significant at the 90 % level	negative correlation	NA

Appendix I-C
WSU Pullman Spreadsheet With Regional Data

WSU Exhibit A-Clopyralid and Picloram Residues in Feedstocks and Compost 10-29-01

Sample	Date	Sample type	Description	Bioassay*	Clopyralid**	Picloram**
Washington State University composts						
ARU 1W	10/11/01	compost	WSU bedding compost product	started 10/15	96	ND
ARU 2W	10/11/01	compost	WSU bedding compost product	started 10/15	169	ND
ARU bedding 4E	7/31/01	compost	WSU bedding compost product	fail	ND(@10ppb)	ND(@10PPB)
ARU bedding 5E	7/31/01	compost	WSU bedding compost product	fail	ND(@10ppb)	ND(@10PPB)
11E	5/8/01	compost	typical WSU compost	fail	206 ppb	38 ppb
4E	5/8/01	compost	typical WSU compost	fail	39 ppb	18 ppb
5E	5/8/01	compost	typical WSU compost	fail	25 ppb	3 ppb
6E	5/8/01	compost	typical WSU compost	fail	18 ppb	3 ppb
7E	5/8/01	compost	typical WSU compost	fail	120 ppb	72 ppb
8E	5/8/01	compost	typical WSU compost	fail	184 ppb	44 ppb
New bedding 2	3/29/01	compost	WSU bedding compost product	fail	66 ppb	5 ppb
KDC-ISO bedding	3/14/01	compost	WSU bedding compost product, isolated from dairy	fail	16 ppb	70 ppb
New bedding	3/12/01	compost	WSU bedding compost product	fail	102 ppb	25 ppb
KDC bedding 2	3/9/01	compost	WSU bedding compost product, isolated from dairy	fail	15 ppb	250 ppb
2W2	1/5/01	compost	typical WSU compost (not same as 2W run on 10/27/00)	fail	3.8 ppb	31 ppb
pea slab	1/5/01	compost	WSU compost that first caused problems/dried and screened for lab (1999 hay)	fail	11.2 ppb	500 ppb
2W	10/27/00	compost	typical WSU compost	fail	7 ppb	24 ppb
Washington State University feed stocks						
NBC manure	10/11/01	manure	WSU beef cattle manure	started 10/15	ND	ND
KDC manure	7/31/01	manure	WSU dairy	fail	3ppb	ND
KDC manure	3/14/01	manure	WSU dairy	fail	6 ppb	45 ppb
timothy hay	7/18/01	hay	purchased by WSU, for vet clinic from Potlatch, ID	fail	9 ppb	ND
timothy hay	6/6/01	hay	purchased by WSU from Yakima, WA	fail	67 ppb	ND
ARU straw 4E	7/31/01	straw	animal research unit straw used in row 4E	fail	ND	12
KDC straw 2 string	7/31/01	straw	new source of straw purchased by WSU for dairy	pass	ND	ND
vet clinic straw	6/12/01	straw	purchased by WSU, for vet clinic	NA	ND	ND
KDC straw	3/13/01	straw	purchased by WSU, for dairy	fail	14 ppb	ND
greenhouse waste	6/12/01	potting mix	waste from WSU campus greenhouse, predominately potting mix	NA	7 ppb	ND

Appendix I-C
WSU Pullman Spreadsheet With Regional Data

Other Composts						
Coyne Compost	8/28/01	compost	compost from Whatman County, Horvander Park	pass	NA	NA
Johnson Compost	8/28/01	compost	Chicken manure and bluegrass compost	fail	NA	NA
Latah Sanitation Compost	8/10/01	compost	Compost from Latah Sanitation, Moscow, ID - yard waste compost	fail	NA	NA
Other agricultural products that enter composting stream						
Gardener's 2001 horse manure	8/10/01	manure	horse manure from 2001, PCEI gardener	fail	NA	NA
PCEI horse manure	7/30/01	manure	horse manure from 2000, PCEI gardener	fail	67 ppb	132 ppb
G4 timothy hay	10/11/01	hay	timothy hay - Princton, ID	started 10/15	421	ND
G5 timothy hay	10/11/01	hay	timothy hay - Princton, ID	started 10/15	446	ND
Gardener's hay	8/10/01	hay	hay PCEI gardener is feeding to horses (source was changed from previous year)	pass	NA	NA
Gardener's horse bedding	8/10/01	straw	horse bedding, PCEI gardener	fail	NA	NA
PCEI straw mulch	7/30/01	straw	chopped straw used as mulch, waste from erosion blanket manufacturing	fail	ND	ND
Garden Soils that did not get WSU compost						
Bunzel garden soil	8/2/01	soil	garden soil treated with locally purchased manure	fail	3 ppb	2 ppb
Olesen garden soil	8/2/01	soil	garden soil treated with manure from owners cows	fail	4 ppb	18 ppb

*Bioassay at WSU with pinto bean, plants with herbicde damage symptoms fail bioassay
**Analytical test by Anatek (EPA 8151modified)
ND = below practical limit of quantition of 1ppb (unless noted)

Appendix I-D

Sampling Plan for Testing of Clopyralid and Plant Toxicity in Compost and Related Materials

Date: February 25, 2002

Prepared by: Clayton Group Services

BACKGROUND

Newspapers have reported plant deformations due to compost containing clopyralid, a chlorinated herbicide. The half-life reported by the manufacturer, Dow AgroChemicals, is 25 days, but it appears to persist longer in compost.

This plan for a pilot study serves to coordinate sampling and testing of compost and related matrices for plant toxicity and the presence of clopyralid.

Recognition of Limitations Statement

This pilot study is not statistically representative. The pilot study will screen for clopyralid in a variety of products and materials and for plant response to those materials. Validation of analytical methods or results will not be a part of this study. This study does not partially or fully account for other factors that may affect the results, such as the presence of other herbicides including picloram, synthetic organic compounds, and heavy and other metals, other elements, phosphorus, nitrogen, potassium, ammonia, cyanides, carbon ratio, ash, density, pH, secondary and micro-nutrients, mineral composition in products, and differences between laboratory methodologies.

Contacts

Table 1 -The Key Contacts

Name	Agency/Company	Project Role	Phone Number
Josh Marx	King County Solid Waste Division	Overall coordination within King County	206 296 4429
Venetia Runnion	Clayton Group Services	Oversight of sampling	206 763 7364
Sonya Manejkowski	Clayton Group Services	Sample collection, prep, and submittal to labs	206 763 7364
Barb Faville	Clayton Group Services	Sample collection, prep, and submittal to labs	206 763 7364

George Perry	King County Hazardous Waste Division	Quality Assurance	206 263 3083
Mike Long	King County Solid Waste Division	Primary contact for Clayton Group Services	206 296 4416
Gary Westburg	Morse Analytical	Testing of compost and grass clippings for clopyralid	916 481 3141
Mike Pearson	Anatek	Testing of all matrices for clopyralid	208 883 2839
Tom Watson	STL Seattle	Testing of all matrices for clopyralid	253 992 2310
Andy Bary	WSU, Puyallup	Plant bioassays	253 445-4588
Dan Caldwell	WSU, Pullman	Plant bioassays	509 335 7514
Fritz Grothkopp	King County Environmental Lab	Contact for decontamination of sampling equipment	206 684 2327
Fran Sweeney	King County Environmental Lab	Plant bioassays data review	206 684 2358

DATA QUALITY

Data Use

Data are being collected to address the following questions:

- Is clopyralid present in the tested materials at sufficient levels to cause observable toxicity in plants?
- Is there a correlation between clopyralid concentrations and the results of the bioassay for the tested materials?
- How comparable are the data being generated from different participating laboratories?

Data should be of sufficient quality to support the following data uses:

- Make decisions regarding destination and use of various compost, mulch and topsoil products and organic waste streams such as foodwaste.
- Provide input to regulatory process addressing uses of clopyralid.
- Provide an indication as to the usability and comparability of clopyralid data generated from different participating laboratories.

There are no Standard Reference Materials (SRM) available at this time for clopyralid. This diminishes the ability of all involved in this work to truly discern data quality. Therefore, a key component to understanding data comparability is the plant bioassay results. Bioassay and clopyralid data will be compared. A positive bioassay, one showing harmful effects, is expected with clopyralid results of approximately 3 parts per billion (ppb) or higher. However, the Lowest Observed Effect Concentration (LOEC) is still to be defined, and there are indications that the LOEC may be as low as 1 ppb. The effects of other chemicals that may result in observable harmful effects in plant bioassays

will generally not be evaluated as part of this pilot study. A limited amount of picloram data will be collected to possibly aid in data interpretation.
King County considers a finished material that produces a positive effect in the plant bioassay as inappropriate for plants. A feedstock that produces a positive effect in the laboratory may be inappropriate for composting. King County may submit the findings of this pilot study to regulatory agencies or others addressing uses of clopyralid in the State of Washington.

Before data use, King County Solid Waste Division (KCSWD) will review data packages from each laboratory. Their review will be based on EPA data review procedures and method performance criteria in Method 8151A.

Specific data quality objectives

Specific data quality objectives are summarized in the narrative below. Quantitative criteria are contained in Tables 2 and 3 below. Picloram data will be collected for information purposes only. No specific data quality objectives are proposed for picloram.

Precision
The precision of the clopyralid testing will be evaluated through the use of single blind sample replicates provided by the sampler. Laboratories will not be notified which samples are replicates. This approach will be used for bioassay sample precision as well.

The Relative Percentage Difference (RPD) will be calculated between clopyralid detections from participating laboratories by KCSWD. A consistent RPD of greater than 66.7 % between laboratories will be evaluated by reviewing data packages and comparing differences in laboratory methodology. Findings will be reported to the project team.

Example Calculation:

Equation: RPD = (result1 – result2/average result) X 100.

Data: *result 1 = 1*
result 2 = 2

RPD =(2 – 1/1.5) X 100 = 66.7%

Bias
Plant bioassays are performed with both positive and negative controls. These will serve as a measure of bias for this test.

Laboratories will perform method blanks and matrix spikes. In addition, surrogate compound recoveries will be reported for each sample. Recoveries should be within control limits specified in Table 3.

Completeness
Samples analyzed within holding times and determined to be useable are considered complete. Due to the small size of this project it is anticipated that data will be 95% or greater complete.

Comparability
The following data sets will be evaluated for comparability:

- clopyralid data and plant bioassay data
- clopyralid data between laboratories

There is a significant on-going effort by agencies and industry studying clopyralid and plant toxicity in feedstocks and products. Overlap in testing laboratories for both plant bioassays and clopyralid will aid in interpreting the entire body of data.

Data review will be conducted by KCSWD using the following tables as guideline criteria. Overall data quality from each laboratory will be evaluated. An occasional exceedance of a QC criterion is to be expected. However, consistent difficulties in meeting QC specifications will be noted and limitations may be placed on the use of some data. The archiving and freezing of samples allows for re-submittal and testing if required.

Table 2 -Summary of Data Quality Criteria – Bioassay

QC Sample or Criteria	Control Limits
Bioassay, positive control	observed effect
Bioassay, negative control	no observed effect

Table 3- Summary of Data Quality Criteria – Clopyralid

QC Sample or Criteria	**Control Limits**
Method Blank	< reported detection limit
Laboratory Control Sample	50-135 % recovery
Surrogate Recovery	50-135 % recovery
Replicate (single blind)*	RPD of 66.7 % or less
Matrix Spike (MS) / Matrix Spike Duplicate (MSD)	50–130 % recovery
Matrix Spike (MS) / Matrix Spike Duplicate (MSD)	RPD of 66.7 % or less

**To be discussed by data reviewers and users. As results (from different labs) approach the detection limit, RPD is more likely to exceed 66.7%. A consistent bias will place limitations on data use.*

Laboratory Quality Control

The following QC samples are required for each batch of samples submitted to a laboratory.

- Method blank

- Matrix spike for each matrix – i.e. manure, compost and topsoil are three separate matrices. Analysis of a matrix spike (MS) and matrix spike duplicate (MSD) will demonstrate method accuracy and precision and monitor matrix interference's.
- Routine laboratory QC such as control samples
- Surrogate recoveries

Laboratory Data Packages

In addition to data summaries, data packages are to be provided by the laboratory. These should include chromatograms and sufficient information to reproduce the data work-up and correctly calculate reported results. The data package must include the following:

- Bench sheets.
- Percent moisture of samples which are to be used to calculate and report all results on a dry weight basis.
- Concentration and dilution factors.
- Documentation of any cleanups performed and the samples that received the cleanups. QC samples must be processed through all cleanup steps.
- Instrument chromatograms, results of check standards, continuing calibrations, and regression results.
- Documentation of deviation from methods and any analytical difficulties encountered.
- Surrogate identities and recoveries.
- Laboratory control limits for all QC samples and instrument calibration. Instrument calibration includes initial and continuing calibration.
- Chain of custody forms.

More information is included in the Instructions to the Laboratory form.

All samples are to be handled and analyzed under laboratory chain of custody procedures, with each employee with custody listed.

Laboratories

Washington State University (Pullman and Puyallup) will perform all plant bioassays. Samples will also be sent to Anatek, which can obtain a 1 ppb detection limit for clopyralid in all matrices of interest. This will aid in comparing clopyralid results between materials. Either STL Seattle laboratories or Morse Analytical will perform a second clopyralid test. The second analysis will aid in understanding overall data comparability.

An analytical detection limit of 1 ppb is desirable for clopyralid. The Lowest Observed Effect Concentration (LOEC) for the plant bioassays approaches 1 ppb. Detection limits may be higher than 1 ppb in some participating laboratories for difficult matrices such as manure or evergreen mulches. A summary of participating laboratories and their matrix dependent detection limits is contained in Table 4.

Table 4 - Participating laboratories and a summary of their capabilities.

Laboratory	Tests Performed	Method Used	Stated DL	Surrogates	Grinding	Lab sample size	Drying temperature	How Stated Detection Limits Were Set
Anatek	Testing of all matrices of interest for clopyralid	In house. Modified EPA 8151	1 ppb, may be higher in difficult matrices	Tetrachloro (m)-xylene; 5- Bromo-nicotinic acid	Grinds feedstock not compost	50 grams wet	105° C	EPA's SW846; 7 replicates of extraction
Morse Analytical	Testing of compost and grass clippings for clopyralid	Dow, modified for lower detection limits	1 ppb	Spikes with clopyralid	Grinds compost and feedstock	10-20 grams	110-120°C	Recoveries (70-120%) at 1 ppb with clean control sample
STL Seattle	Testing compost, may do feedstock materials	In house. Modified EPA 8151A	2-5 ppb in soil; 2 ppb in solid method blanks	2,4 - Dichloro phenyl acetic acid	Grinds feedstock not compost	15 grams	EPA SW methods temperature	MDL study
WSU (Pullman and Puyallup)	Plant bioassays	In house.	NA	NA	NA	NA	NA	Positive or negative

SAMPLING

All samples will be collected and analyzed under chain of custody procedures. A digital photograph of the original material and a filled sample container will be taken. Sample homogeneity within purchased materials is assumed because the pile that has undergone thorough mixing during the compost process. Some mixing will occur as the contents are prepared for sampling.

Samples will be taken using the following procedure. An unidentified pickup truck will be used to purchase bulk materials. The bed will be lined in plastic sheeting or a new tarp and the loaded material will be covered in an effort to prevent cross-contamination. For bagged material, plastic sheeting or plastic bags, or a new tarp will be used as a barrier and cover for each bag transported. To avoid cross-contamination, separate barriers will be used for each material purchased.

The following cleaning and sampling protocol is closely based on procedures provided by the Washington State Department of Ecology. Approximately 15 sets of identical stainless steel containers and spoons will be submitted for cleaning prior to sampling. The equipment will be cleaned according to this protocol:

1. Wash with laboratory detergent.
2. Rinse several times with tap water.
3. Rinse three times with distilled / de-ionized water
4. Rinse with high purity acetone or methanol
5. Rinse with ultra high purity hexane
6. Allow to dry, place within aluminum foil, and seal until use.
7. Document decontamination.

Samples will be collected to be as representative as possible. The assumption (for 'representativeness') is that the pile has undergone thorough mixing during the composting process.

Each compost pile to be sampled will be visually marked at five distinct sampling sites. Three approximately equal volume grab samples will be taken from each sampling site, one at the upper 1/3 of the pile, one at the middle 1/3 of the pile and one at the bottom 1/3 of the pile. The minimum number of grabs for each composite will be 15. (Three grabs, each at different depths of the pile, for each of the five sampling sites).

After removing any large twigs or stones, the composite sample will be thoroughly mixed.

All samples will be taken using pre-cleaned stainless steel spoons and pre-cleaned stainless steel containers. One lab will provide organic-free laboratory –cleaned sample jars. Other labs are requiring Ziploc® plastic bags.

All samples will be labeled with a sample number, date, and the sampler's initials. A laboratory Chain-of-Custody will also be completed at the time of sampling. Upon sample collection and proper labeling, samples will be stored in a cooler and maintained at a temperature of 4° C or less until analyzed.

Approximately four to five cups of material will be collected at the three depths in the five horizontal locations to collect an intermediate quantity (the composite) into the stainless steel container to be divided. If that procedure does not yield enough material for the composite, the procedure will be repeated using five different horizontal locations. The cups of material will be mixed manually for a minimum of 1 minute in a stainless steel container. If the material appears to be dry and settling of fines has occurred, mixing will be done for a minimum of 3 minutes. Material that falls out of the stainless steel container will not be placed back into the composite.

The composite material will be divided into portions for the laboratories; each portion is considered to be a "split sample" because it came from the same stainless steel container's composite mixture. When collecting the portions, rotate around the stainless steel container and place material in the Ziploc® bag or glass jar. From the composite, about ½ gallon will be collected for visual identification and archived for KCSWD. Excess air will be pressed out of the Ziploc® bag.

Ten percent (or at least 2 portions) of the composite material will be collected for blind replicate analysis. When a sample is split and sent to multiple laboratories, each of those portions will have a unique number. A wax or permanent ink pen will be used for labeling.

Clayton will archive frozen samples for future analysis (if needed) for no more than one year from sample date. See Table 5 for sample portion size requirements, container information, and storage temperatures. Any remaining material will be used on private property in unincorporated Snohomish County in an R5 (rural) area.

Samplers will wear latex or nitrile gloves and will discard them as necessary to aid in the prevention of cross-contamination. Samplers will avoid touching the materials directly. A new tarp or plastic liner, stainless steel container and scoop, and sample containers will be used for each set of samples.

The sample splits will be placed in plastic zipper bags or the container provided by the laboratory and will be bagged again. It may be necessary to place the Ziploc® plastic bags in a Gladware® or other plastic container for security during shipment. Each plastic bag envelope will be placed in a cooler containing dry ice or blue-ice. The cooler will be sealed, stored in a locked freezer, and shipped the same week to the laboratories. Laboratory delivery dates shall be documented. All shipping papers will be retained.

Table 5 – Sample Analysis Summary

Matrix	Bioassay at WSU	Anatek for clopyralid	Morse for clopyralid	STL Seattle for clopyralid	Blind replicate *	Archive for KCSWD
Compost and grass	X	X	X		X	X
All other matrices	X	X		X	X	X
Field sample size	>½ gallon plastic bag	1 gallon plastic bag	< ½ gallon plastic bag	4 oz glass jar	Same as field sample size	½ gallon plastic bag
Temperature** for shipping and storage	4° C	4° C	Freezing, < 0° C Use dry ice.	4° C	Same as field sample	4° C for transport; freezing for archive

* At least 10% of samples (minimum of 2) will be submitted as blind replicates.
**Temperature in the cooler will approximate desired temperatures.

Table 6 – Product Materials to be sampled, February and March, 2002

Product	Manufacturer	Manufacturing Process	Sampling Location	Sampling Procedure
Bag Compost	Cedar Grove	Made from yard waste	2 retail outlets with rapid product turnover: 1 Nursery (Swanson's or Molbak's) and 1 home improvement store (Home Depot or Lowe's)	Sample each bag separately.
Bulk Compost	Cedar Grove	Made from yard waste	877-764-5748 http://www.cedar-grove.com/	Buy one cubic yard. Use lined pick up truck to transport.
Bulk Compost	Soos Creek	Made from yard waste	Covington (253) 639-0055	Buy one cubic yard. Use lined pick up truck to transport.
Bag Manure	Whitney Farms	Made from manure and animal bedding	1 Nursery Furney's or Molbak's	Buy one bag.
Bulk Manure	Steerco	Made from dairy manure and saw dust	Kent (253) 622-5141	Buy one cubic yard. Use lined pick up truck to transport
Bulk 2 Way Mix Topsoil	Pacific Top Soil	Made of Sandy loam soil and compost, 1/2in. screen	800-884-7645 Bothell http://www.pacifictop soils.com	Buy one cubic yard. Use lined pick up truck to transport.
Bulk Enviro Mix	Pacific Top Soil	Made of Pacific Garden Mulch &	800-884-7645 Bothell	Buy one cubic yard. Use lined pick up truck

		sand, 1/2in screen, 1/3 sand	http://www.pacifictop soils.com	to transport.
Straw	Retail sale of farm straw	Made of Oat Straw from farms in E. Washington, Idaho, or Montana	Grange 425-392-6469 Carnation Lumber Rocking E Iron Horse Del's Farm Supply 253-833-3550	Buy one bale. Use lined pick up truck to transport.
Christmas Trees	Rainier Wood	Chipped (if seasonally available)	Cedar Falls SW Facility in Kent or Fall City	Buy one cubic yard. Use lined pick up truck to transport.

~~Table 7 – Feedstocks to be sampled by May, 2002~~

~~Feedstock~~	~~Manufacturer~~	~~Sampling Location~~	~~Sampling Procedure~~
~~Pre Consumer Vegetative Commercial Food~~	~~NA~~	~~Cedar Grove~~	~~Buy one cubic yard. Use lined pick up truck to transport.~~
~~Post Consumer Residential Food~~	~~NA~~	~~Cedar Grove~~	~~Buy one cubic yard. Use lined pick up truck to transport~~
~~Post Consumer Commercial Food~~	~~NA~~	~~Cedar Grove~~	~~Buy one cubic yard. Use lined pick up truck to transport~~

~~Note: NA = Not Applicable~~

All samples are to be collected under chain of custody procedures. Chain of custody is defined as meeting one or more of the following conditions:

- The sample is in custodian's view.
- The sample is in a secure container, a locked vehicle or secured sample cooler.
- The sample is in a secure area, such as a limited access laboratory.

Additionally, each employee of the same agency/company is considered separate and must be listed on the chain of custody.

DOCUMENTATION

Samples are to be collected using Chain of Custody forms from the laboratories, and the Sampling Documentation form included in this document.

Original correspondence, letters, shipping papers and phone logs must be saved and transmitted to Josh Marx. Mike Long and Josh Marx shall be copied on all internal and external communication that occurs regarding this project. Original data packages will be provided to Josh Marx. Upon request or at project completion, all data will be available to Josh Marx.

All participating agencies and companies are to maintain strict confidentiality on any matter related to this sampling and testing effort.

This work is being conducted under Clayton contract T01444T.

Sampling Documentation

One page per source pile or bag

Name of Product:
Date of Product Purchase:
Business Name and Address:
Business Contact, if Applicable:
Date of Sample Collection:
Approximate Time of Collection:
Samplers Present (full name):
Form Filled Out By:
Location of actual sampling:

Sample Description

Pile or Bag Sample #	Matrix	Estimated date of Manufacture	Photo	Environmental conditions (weather)	Comments (moist or dry with settled fines)

Sample Collection

Split Sample #	Plan sampling procedure (≥15 collection points) used?	Container type	Laboratory Destination	Comments

Instructions to Laboratory - Herbicides

Confidentiality
All participating agencies and companies are to maintain strict confidentiality on any matter related to this sampling and testing effort.

Contacts
Direct all questions regarding this project to Venetia Runnion, Barb Faville, or Sonya Manejkowski at Clayton Group Services, 4636 E. Marginal Way South, Seattle, Washington 98134, phone number 206 763-7364, fax 206 763-4189.

Testing Instructions
Test for parameters indicated on the Chain of Custody. If clopyralid is requested report picloram as well, if normally included by your laboratory in the method target list. Each employee with custody of the samples must be listed on the Chain of Custody. Always perform a percent moisture test on the sample and report total solids. Report clopyralid results in parts per billion (ppb), on a dry weight basis.

QC Requirements and Reporting Data
Analyze and report method blank(s) and laboratory control samples per standard lab SOP. Perform a matrix spike/matrix spike duplicate for each matrix. Thus, a MS/MSD would be analyzed for compost (or soil), and for manure, and for straw, etc. After spiking, let the matrix spike "rest" for at least one hour prior to proceeding with testing.

Report a data summary to Venetia Runnion within 21 days of sample receipt. Include a data package that is legally defensible. It must contain sufficient information to reproduce the data work-up and correctly calculate reported results. The data package must include the following:

- Bench sheets, concentration and dilution factors.
- Documentation of any cleanups performed and the samples that received these cleanups.
- QC samples must be processed through all cleanup steps.
- Instrument chromatograms, results of check standards, continuing calibrations, and regression results.
- Documentation of deviation from methods and any analytical difficulties encountered.
- Chain of custody forms.
- Surrogate identities and recoveries.
- Laboratory control limits for all QC samples and instrument calibration. Instrument calibration includes initial and continuing calibration.

Sample Storage
All samples should be retained by the laboratory and properly stored until written permission is granted for disposal or sample return.

Billing
Invoices should be sent to Venetia Runnion, Clayton Group Services.

Instructions to Laboratory - Bioassay

Confidentiality
All participating agencies and companies are to maintain strict confidentiality on any matter related to this sampling and testing effort.

Contacts
Direct all questions regarding this project to Venetia Runnion, Barb Faville, or Sonya Manejkowski at Clayton Group Services, 4636 E. Marginal Way South, Seattle, Washington 98134, phone number 206 763-7364, fax 206 763-4189.

Testing Instructions
Test for parameters indicated on the Chain of Custody. Each employee with custody of the samples must be listed on the Chain of Custody. Always perform a percent moisture test on the sample and report total solids. Tests should be started within 14 days of receipt.

QC Requirements and Reporting Data
Analyze and report a positive and negative control sample per standard lab SOP.

Report a data summary to Venetia Runnion within 21 days of sample receipt. Include a data package that is legally defensible. It must contain sufficient information to reproduce the data work-up. The data package must include the following:

- Bench sheets, concentration and dilution factors. Daily greenhouse conditions.
- QC samples must be processed through all steps.
- The results of any routine monitoring instrument calibration, such as thermometers.
- Documentation of deviation from methods and any analytical difficulties encountered.
- Chain of custody forms.
- Laboratory control limits for all QC samples and instrument calibration.
- A copy of the procedure used.

Sample Storage
All samples should be retained by the laboratory and properly stored until written permission is granted for disposal or sample return.

Billing
Invoices should be sent to Venetia Runnion, Clayton Group Services.